7 ANSWER 1 OF 2 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AN 2003:374855 SCISEARCH
GA The Genuine Article (R) Number: 672BT
TI Characterization of the role of the divalent metal ion-dependent transcriptional repressor MntR in the virulence of Staphylococcus aureus
AU Ando M; Manabe Y C; Converse P J; Miyazaki E; Harrison R; Murphy J R; Bishai W R (Reprint)
CS Johns Hopkins Univ, Sch Med, Ctr Tuberculosis Res, Dept Med, Div Infect Dis, 424 N Bond St, Rm 112, Baltimore, MD 21205 USA (Reprint); Johns Hopkins Univ, Sch Med, Ctr Tuberculosis Res, Dept Med, Div Infect Dis, Baltimore, MD 21205 USA; Johns Hopkins Univ, Bloomberg Sch Publ Hlth, Dept Int Hlth, Div Dis Control, Baltimore, MD 21205 USA; Boston Univ, Sch Med, Dept Med, Sect Mol Med, Boston, MA 02118 USA; Adv Microbial Solut, Milford, MA USA
CYA USA
SO INFECTION AND IMMUNITY, (MAY 2003) Vol. 71, No. 5, pp. 2584-2590. Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0019-9567.
DT Article; Journal
LA English
REC Reference Count: 40
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB **DtxR**-type metal ion-dependent repressors, present in many bacterial pathogens, may regulate expression of virulence genes such as that encoding diphtheria toxin. SirR, a **DtxR** homologue initially identified in Staphylococcus epidermidis, governs the expression of the adjacent sitABC operon encoding a putative metal ion ABC transporter system. We identified a sirR homologue, mntR, in Staphylococcus aureus and demonstrated by gel shift assay that the corynebacterial repressor **DtxR** binds to the S. aureus mntABC operator in the presence of Fe2+ or Mn2+. Since a mutant **DtxR**, **DtxR**(E175K), functions as an iron-independent hyperrepressor in certain settings, we constructed a heterodiploid S. aureus strain expressing **dtxR** (E175K) from the native mntR promoter. Transcription of the S. aureus mntABC operon was repressed in the presence of Fe2+ or Mn2+ in wild-type and heterodiploid S. aureus strains. Under metal ion-limiting conditions, mntABC transcription was reduced but not abolished in S. aureus isolates expressing **dtxR**(E175K) compared with an isogenic control, suggesting that **DtxR**(E175K) binds the S. aureus MntR box in vivo. Under all conditions tested, mntABC transcription in the **dtxR**(E175K)-expressing strain was reduced relative to the isogenic control, indicating that **DtxR**(E175K) function was constitutively active. In the mouse skin abscess model, **dtxR**(E175K)-expressing S. aureus **recombinants** showed significantly reduced CFU levels compared with the isogenic wild-type control. We conclude that the S. aureus MntR box is recognized by corynebacterial **DtxR** proteins and thus belongs to the **DtxR** family of metal-dependent operator sites. Moreover, constitutive repression by **DtxR**(E175K) reduces the virulence of S. aureus in the mouse skin abscess model.

L7 ANSWER 2 OF 2 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
AN 2001-335683 [35] WPIDS
DNC C2001-103677
TI New vaccines containing avirulent or attenuated microbes, useful for enhancing protective immunity against, or for attenuating or reducing the severity of an infection or disease caused by bacteria, e.g. **Mycobacterium** tuberculosis.
DC B04 D16
IN HARRISON, R J; MURPHY, J R; O'LEAR, E
PA (ADMI-N) ADVANCED MICROBIAL SOLUTIONS CORP
CYC 94
PI WO 2001030384 A1 20010503 (200135)* EN 54p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ

NL OA PT SD SE SL SZ TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM
DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC
LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE
SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2001011017 A 20010508 (200149)
ADT WO 2001030384 A1 WO 2000-US29231 20001023; AU 2001011017 A AU 2001-11017 20001023
FDT AU 2001011017 A Based on WO 200130384
PRAI US 1999-161292P 19991025; US 1999-161193P 19991022
AN 2001-335683 [35] WPIDS
AB WO 200130384 A UPAB: 20010625
NOVELTY - A virulent or opportunistic prokaryote (I) in which metal ion-independent gene regulation confers a growth or an infectious advantage, is new. The prokaryote is transformed with a DNA molecule encoding a dominant, metal ion-independent repressor protein or a partially metal ion-independent repressor protein. The DNA molecule is expressed in the prokaryote.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a composition comprising (I);

(2) an isolated and purified DNA molecule (II) consisting essentially of a sequence encoding a metal ion independent or a partially metal ion independent diphtheria toxin repressor (**DtxR**) or its homologue;

(3) a **recombinant** DNA molecule containing a constitutive promoter element in operable association with (II);

(4) a **recombinant** vector comprising a promoter element in operable association with (II);

(5) a method of enhancing protective immunity against infection or disease caused by the opportunistic or virulent prokaryotic pathogen comprising administering to an animal the composition; and

(6) a method of attenuating or reducing the severity of an infection or disease caused by the opportunistic or virulent prokaryotic pathogen comprising administering to an animal the composition (I).

ACTIVITY - Antibacterial. Forty-eight 6-8-week old BALB/c mice were infected by tail vein injection with 2 multiply 105 organisms of wild type M. tuberculosis or M. tuberculosis **DtxR**(E175K). Bacterial infection was monitored over a 119-day period. Results showed that mice infected with wild type M. tuberculosis began to lose weight beginning at 13 weeks, while the M. tuberculosis **DtxR**(E175K)-infected animals initially gained weight, then maintained stable weights for the duration of the experiment.

MECHANISM OF ACTION - Vaccine.

USE - The composition is useful for enhancing protective immunity against infection or disease caused by the opportunistic or virulent pathogen in an animal. The composition is also useful for attenuating or reducing the severity of the infection or disease in animal. In particular, the animal is a human (all claimed).

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ANSWER 1 OF 5 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AN 2003:412508 SCISEARCH
GA The Genuine Article (R) Number: 677VD
TI Trace elements and nitric oxide function
AU Marletta M A (Reprint); Spiering M M
CS Univ Calif Berkeley, Dept Chem, Berkeley, CA 94720 USA (Reprint); Univ Calif Berkeley, Dept Mol & Cell Biol, Berkeley, CA 94720 USA; Univ Michigan, Dept Med Chem, Ann Arbor, MI 48109 USA
CYA USA
SO JOURNAL OF NUTRITION, (MAY 2003) Vol. 133, No. 5, Supp. [1], pp. 1431S-1433S.
Publisher: AMER INST NUTRITION, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA.
ISSN: 0022-3166.
DT Article; Journal
LA English
REC Reference Count: 23
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Nitric oxide (NO) has emerged over the last 15 y as a mammalian metabolic intermediate that is involved in the regulation of critical physiological functions such as blood vessel homeostasis, neuronal transmission and host response to infection. NO is synthesized by the enzyme nitric oxide synthase, which converts the amino acid L-arginine to citrulline and NO. NO functions in biological systems in two very important ways. First, it has been found to be a messenger by which cells communicate with one another (signal transduction), and second, it plays a critical role in the host response to infection. In this second function, it appears that the toxic properties of NO have been harnessed by the immune system to kill or at least slow the growth of invading organisms. The nonspecific chemical reactivity with key cellular targets is responsible for this action. In signaling, NO directly activates the enzyme soluble guanylate cyclase (sGC). Once activated, sGC converts GTP to cGMP and pyrophosphate. The cGMP formed is responsible for the well-documented actions of NO such as blood vessel dilation. With the initial discovery of NO signaling, several important questions emerged that centered largely on the issue of how a signaling system functions when the signaling agent is chemically reactive (short lived), highly diffusible and toxic. Critical, especially in signaling, are the control of NO biosynthesis and interaction with the biological receptors at a concentration that will not harm the host. Why did Nature choose NO for the roles it has? That question engenders only speculation. How does NO work (i.e., what does NO do, and how does it do it without harm yet with specificity)? Answers to these questions can now be offered as the molecular level details emerge to form an interesting picture.

L2 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2003:257144 CAPLUS
DN 138:364487
TI The deactivation of diphtheria toxin repressor by nitric oxide
AU Spiering, Michelle Marie
CS Univ. of Michigan, Ann Arbor, MI, USA
SO (2002) 169 pp. Avail.: UMI, Order No. DA3058047
From: Diss. Abstr. Int., B 2003, 63(7), 3286
DT Dissertation
LA English
AB Unavailable

L2 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1995:317933 CAPLUS
DN 122:232508
TI Molecular cloning, DNA sequence analysis, and characterization of the Corynebacterium diphtheriae dtxR homolog from Brevibacterium lactofermentum
AU Oguiza, Jose A.; Tao, Xu; Marcos, Ana T.; Martin, Juan F.; Murphy, John R.

CS Fac. Biology, Univ. Leon, Leon, 24071, Spain
SO Journal of Bacteriology (1995), 177(2), 465-7
CODEN: JOBAAY; ISSN: 0021-9193
PB American Society for Microbiology
DT Journal
LA English
AB A homolog of the Corynebacterium diphtheriae dtxR gene was isolated from Brevibacterium lactofermentum. The product of the B. lactofermentum dtxR gene was immunoreactive with polyclonal anti-DtxR antibodies and functioned as an iron-activated repressor capable of regulating the expression of .beta.-galactosidase from a diphtheria tox promoter/operator transcriptional fusion in recombinant Escherichia coli. The extents of induction by increasing concns. of the chelator 2,2'-dipyridyl were identical in cells expressing DtxR from either C. diphtheriae or B. lactogermentum.

L2 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1994:237363 CAPLUS
DN 120:237363
TI Cloning, sequence, and footprint analysis of two promoter/operators from Corynebacterium diphtheriae that are regulated by the diphtheria toxin repressor (DtxR) and iron
AU Schmitt, Michael P.; Holmes, Randall K.
CS Dep. Microbiol., Uniformed Serv. Univ. Health Sci., Bethesda, MD, 20814, USA
SO Journal of Bacteriology (1994), 176(4), 1141-9
CODEN: JOBAAY; ISSN: 0021-9193
DT Journal
LA English
AB DtxR is an iron-dependent sequence-specific DNA-binding protein that binds to the tox operator, an inverted-repeat nucleotide sequence located upstream from the diphtheria toxin gene. In this study, two addnl. iron-regulated promoter/operator sequences (IRP1 and IRP2) that are controlled by DtxR were cloned from the chromosome of Corynebacterium diphtheriae and characterized. Operon fusions to lacZ were used to analyze expression from IRP1 and IRP2 in Escherichia coli. Transcription from both promoters was strongly repressed in high-iron medium in the presence of the cloned dxtR gene; however, transcription in the absence of dtxR was 50- to 100-fold greater, regardless of the iron concn. Purified DtxR altered the electrophoretic mobility of DNA fragments carrying IRP1 and IRP2, and the nucleotide sequences of the two promoter/operator regions indicated that they are both homologous with the tox operator. DtxR protected an approx. 30-bp region of both IRP1 and IRP2 from DNase I digestion. A 19-bp consensus DtxR-binding site was derived from a comparison of the various DtxR-regulated operator/promoter sequences. Footprinting expts. using hydroxyl radicals and di-Me sulfate demonstrated that DtxR interacted with these operators in a sym. manner, probably as a dimer or multimer. The deduced amino acid sequence of an open reading frame (ORF1) located downstream from IRP1 was homologous with a family of periplasmic proteins involved in iron transport in gram-neg. bacteria and with the ferrichrome receptor, FhuD, from Bacillus subtilis. These findings suggest that ORF1 encodes a membrane-assocd. lipoprotein that may serve as the receptor for a ferric-siderophore complex in C. diphtheriae.

L2 ANSWER 5 OF 5 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1993:73829 BIOSIS
DN PREV199395038329
TI Transcription analysis and nucleotide sequence of tox promoter/operator mutants of corynebacteriophage beta.
AU Krafft, Amy E.; Tai, Shih-Peng S.; Coker, Christopher; Holmes, Randall K. (1)
CS (1) Dep. Microbiol., Uniformed Services Univ. Health Sci., Bethesda, Maryland 20814 USA
SO Microbial Pathogenesis, (1992) Vol. 13, No. 2, pp. 85-92.

ISSN: 0882-4010.
DT Article
LA English
AB The production of diptheria toxin (DT) by Corynebacterium diphtheriae C7(beta) is transcriptionally regulated by the iron-dependent **diptheria toxin repressor**, DtxR. Transcription of the tox gene was studied in wild-type C. diphtheriae C7(beta) and in lysogens carrying mutants of beta that determine insensitivity to inhibition of DT production by iron. Under low iron conditions in all strains, tox-specific mRNA appeared and DT production began during late-log phase, and they increased to maximal levels at stationary phase. Under high iron conditions, tox-specific mRNA and DT production were strongly repressed in C7(beta) but only partially repressed in C7(beta-tox-202) and C7(beta-tox-201). Under high and low iron conditions, DT production and tox-specific mRNA levels were greater in C7(beta-tox-201) and C7(beta-tox-202) than in wild-type C7(beta). Addition of iron or rifampicin to low iron cultures of C. diphtheriae C7(beta) repressed tox-mRNA production promptly and with a similar time course. In contrast, repression of tox-mRNA synthesis in C. diphtheriae C7(beta-tox-201) occurred promptly after addition of rifampicin but more slowly after addition of iron. Nucleotide sequence analysis revealed single G to A mutations at positions -47 and -48, within the preferred '-10' sequence of the tox promoter, in beta-tox-201 and beta-tox-202, respectively. The single nucleotide substitutions in the tox-201 and tox-202 regulatory alleles, therefore, have pleiotropic effects, causing increased activity of the promoter and partial resistance of the operator to iron-dependent repression.

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